SINGLE LABORATORY VALIDATION OF AN HLPC-RID METHOD FOR SUGAR ANALYSIS IN SOFT DRINKS

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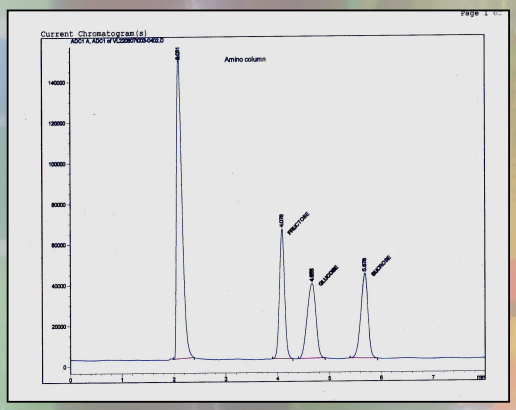
INTRODUCTION

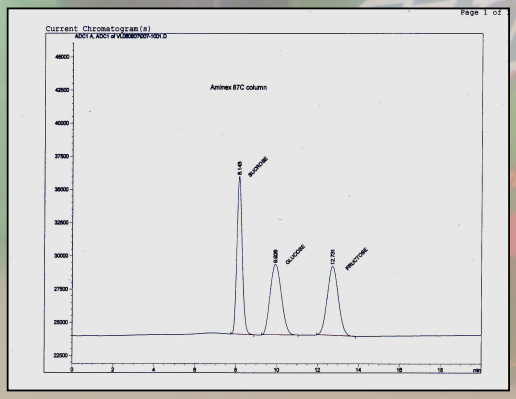
According to Uruguayan Government Regulations, imported sugar to be manufactured is under technical control and does not pay the 35 % tax to ordinary sugar imports. Our Organization, Technological Laboratory of Uruguay is in charge of this control.
This is why we need to perform a fast and reliable method for sugar content analysis in the final products.
HPLC-RID is an excellent tool for the analysis of the sugars present in foodstuffs.
The method used can separate and quantify simultaneously fructose, glucose and sucrose. Fructose and glucose must be analyzed with sucrose because in some matrices we can find sucrose inversion.

EXPERIMENTAL

Samples are degassed and filtered by 0.2 um before the injection

EQUIPMENT:
HPLC Hewlett Packard 1050 with Refractive Index Detector HP 1047A and Automatic Injector Agilent 1313A
COLUMNS:
Biorad Aminex HPX-87C (ion-exchange resin column) 300 mm x 7.8 mm
Phenomenex NH2 amino bonded phase 25cm x 4.6 mm x 5um
HPLC CONDITIONS:
Aminex column:
mobile phase: 100% water
flow: 0.6 ml/min
oven temperature: 80 ºC
NH2 column:
mobile phase: water:acetonitrile 23:77
flow: 1.5 ml/min
oven temperature: 25 ºC





VALIDATION REPORT

ACCURACY:
We use as a measure of accuracy the recovery from spiked blank samples (water and diet cola). The diet cola we used has a glucose content of 0.01 g/100ml which is negligible compared with the spike levels.
RECOVERY:
We spiked blanks at three levels for each sugar:
fructose: 10, 5 and 2.5 g/100ml
glucose: 10, 5 and 2.5 g/100ml
sucrose: 15, 10 and 5 g/100ml
Recoveries from cola soft drink:
fructose: 105.9% (RSD=3.2% n=25), glucose: 106.6% (RSD= 3.2% n= 25), sucrose: 92.7% (RSD=2.9% n=18)
Recoveries from water:
fructose: 96.6% (RSD=2.6%) glucose: 99.0% (RSD=2.7%)
sucrose: 98.1% (RSD=3.6%)
From this data we conclude that the positive bias of fructose´s and glucose´s recoveries and the negative bias of sucrose´s recovery are due to sucrose inversion at the acidic pH of the cola beverage.
CALIBRATION CURVE/LINEARITY:
Aminex column:
fructose: 0.2ug to 1200 ug (24 levels) r=0.9997
glucose: 0.2ug to 1200 ug (25 levels) r=0.9999
sucrose: 0.3 ug to 600 ug (13 levels) r=0.9999
NH2 column:
fructose: 5.3 ug to 1200 ug (13 levels) r=0.9997
glucose: 6.3 ug to 1200 ug (13 levels) r=0.9999
sucrose: 100 ug to 1200 ug (15 levels) r=0.9998
DETECTION LIMITS:
fructose: 0.08 ug
glucose: 0.09 ug
sucrose: 0.05 ug
QUANTITATION LIMITS:
We consider as the quantitation limit the lowest point of the calibration curve.
fructose: 0.2ug
glucose: 0.2ug
sucrose: 0.3 ug
PRECISION:
In Aminex column:
RSDr for fructose ranged from 0.1 to 1.3 % (n=2 at each level)
iRSDR for fructose: ranged from 2.8 to 2.9 % at different levels (n=8-9 at each level)
RSDr for glucose ranged from 0.01 to 0.6% (n=2 at each level)
iRSDR for glucose: ranged from 3.0 to 3.4% at different levels (n=8-9 at each level)
RSDr for sucrose ranged from 0.1 to 0.6% (n=2 at each level)
iRSDR for sucrose: ranged from 3.0 to 3.1 % at different levels (n=6-9 at each level)
HORRATr range from 1.0 to 1.2 depending on the sugar and the spike level
SPECIFICITY:
In the conditions described above, both columns can separate the sugars with a good resolution. Depending on the sample, we can work with Aminex or NH2 columns, or both of them in order to confirm identity. Aminex and NH2 columns elute the sugars in a completely different pattern.
SENSITIVITY:
We can quantitate levels higher than 0.2 ug for fructose and glucose and 0.3 ug for sucrose.
RUGGEDNESS:
We are finishing with the study of the precision with NH2 column in order to see if we can use either of the columns with the same precision.
UNCERTAINTY: 2.6 %

CONCLUSIONS

In the conditions described we can control the sugar content of beverages with a good accuracy, precision, sensitivity and specificity. Aminex column has the advantage of the use of 100% water as the mobile phase, this means no use of expensive solvents and no problems with the solvent disposal. Another advantage is for beverages containing sorbitol. This column separates glucose from sorbitol, the ones who coelutes in an NH2 column. As a big disadvantage, Aminex column is 4 times more expensive than NH2 column. NH2 column has the advantage of the price and the possibility of use different solvent composition in order to have better separations.